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Review

Study of a supplement and a genetic test for lymphedema management

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Abstract. Malformations in the lymphatic vasculature, injury, surgery, trauma or toxic damage may lead to swelling of the limbs caused by inefficient lymphatic uptake and flow (lymphedema). Lymphedema can be congenital or acquired. Primary lymphedema is rare and caused by mutations in single genes, secondary lymphedema is more common and caused by a trauma in association with a genetic predisposition. We decided to develop a genetic test that would determine the genetic predisposition to the onset of lymphedema and to predict the course of the disease by analyzing polymorphisms involved in leukotriene B4 (LTB4) synthetic pathway, and variants involved in the onset of secondary lymphedema. There are not many compounds available for the treatment of the negative effects of lymph accumulation, we therefore designed a food supplement based on the hydroxytyrosol, that has anti-oxidant, anti-bacterial and anti-inflammatory activities. (www.actabiomedica.it)

Key words: lymphedema, hydroxytyrosol, leukotriene B4, food supplement

Introduction

Malformations in the lymphatic vasculature, injury, surgery, trauma or toxic damage may lead to lymphedema, a swelling of the limbs caused by inefficient lymphatic uptake and flow (1). Lymphedema is classified as primary when congenital and secondary when acquired (2). Usually, primary lymphedema is determined by a mutation in a single gene, whereas secondary lymphedema is associated with a trauma, but genetic predisposition may be involved.

Since secondary lymphedema is quite common in the population (lymphedema affects 200 million people worldwide and around 3 million people in the United States), we decided to develop a genetic test that would determine the genetic predisposition to the onset of lymphedema and to predict the course of the disease by analyzing polymorphisms involved in leukotriene B4 (LTB4) synthetic pathway, LTB4 is the major mediator of inflammation (3) (Table 1). It promotes lymphatic endothelial cells growth at low concentrations, but causes lymphatic endothelial cell injury at high concentrations (4). We also included variants involved in the onset of secondary lymphedema, in order to predict the predisposition to lymphedema after trauma, surgery or infection.

Since there are not so many compounds available for the treatment of the negative effects of lymph accumulation, we also designed a food supplement based on the hydroxytyrosol (HT), extracted from olive trees. HT is a compound with anti-oxidant, anti-bacterial and anti-inflammatory properties. We previously reviewed in a previous work the promising properties of HT in the treatment of the effect of lymph accumulation by blocking leukotriene B4 generation (5).

Table 1. Polymorphisms that can predispose to secondary lymphedema and/or modulated the clinical course of lymphedema

Gene	Gene function (GeneCards)	rs ID, alleles	Association	Ref.
LTB4R2	Chemotaxis mediation of granulocytes and macrophages	rs1950504, A/G	Enhanced ROS generation/AKT phosphorylation under LTB4 low-dose conditions. Enhanced cell motility under low-dose ligand stimulation	6
ALOX5	Catalyzes the first step in leukotriene biosynthesis and has a role in inflammatory processes	rs4987105, C/T	Lower 25(OH)D3 and 1,25(OH)2D3, and higher C-reactive protein levels in homozygote CC carriers. This may indicate a chronic inflammatory profile. It modulates the response to the leukotriene antagonist montelukast	7
		rs59439148, $del(GGGGGC)_{4/3/2}$/ $del(G)_5C$/$dup(G)_5C$/ $dup(GGGGGC)_{2/3}$	Determination of the expression levels of ALOX5. Two copies of a minor variant of the ALOX5 promoter SP1 tandem repeat polymorphism is associated with increased cysteinyl leukotriene production	8
		rs4769874, G/A	GG genotype is associated with modest increase in body mass index. The A-allele potentiates the expression of ALOX5 and/or the function of FLAP	9
LTA4H	Epoxide hydrolase that catalyzes the final step in the biosynthesis of leukotriene B4	rs17525495, C/T	T allele associated with lower levels of LTA4H. The presence of the T allele significantly increased the proportion of Crohn's patients requiring glucocorticoids	10
		rs1978331, C/T		
MMP2	Metalloproteinase involved in remodeling of the vasculature, angiogenesis, tissue repair, inflammation	rs1030868, G/A	A allele, higher risk of secondary lymphedema	11
		rs2241145, G/C	C, higher risk of secondary lymphedema	11
CEACAM1	Cell-cell adhesion molecule with roles in angiogenesis, modulation of immune response. Inflammasome activity reduction. Blood vessel remodeling through endothelial cell differentiation and migration. Vascular permeability regulation	rs8110904, G/A	A, higher risk of secondary lymphedema	11
		rs8111171, G/T	T, higher risk of secondary lymphedema	11
FOXC2	Transcriptional activator. Involved in the mesenchymal tissue formation	rs199772307, G/A	AA genotype more frequent in lymphatic filariasis patients, influence on the severity of lymphedema	12
		rs34221221, A/G	G allele, increased expression	13

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Table 1 *(continued)***.** Polymorphisms that can predispose to secondary lymphedema and/or modulated the clinical course of lymphedema

Gene	Gene function (GeneCards)	rs ID, alleles	Association	Ref.
TNF	Cellular responses to cytokines and stress. It regulates the immunological response to infections	rs1800629, G/A	High percentage of TNFa homozygotes GG in patients with dermato-lymphangio-adenitis in obstructive lymphedema of lower limbs	14
TLR2	Key role in the innate immune system. It is expressed in macrophages, B lymphocytes, mast cells	rs121917864, C/T	Low percentage of CT heterozygotes and TT homozygotes in patients with dermato-lymphangio-adenitis in obstructive lymphedema of lower limbs	
TLR4	Key role in the innate immune system. It is expressed in macrophages, B lymphocytes, mast cells	rs4986791, C/T	High percentage of CT heterozygotes and TT homozygotes in patients with dermato-lymphangio-adenitis in obstructive lymphedema of lower limbs	
VEGFA	Growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. Induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels	rs699947, C/A	-2578C>A Lower or higher expression	15
		-1154G>A	A allele, lower expression	15
		-460C>T	T allele, increased promoter activity	15
		+405G>C	C allele, lower or higher expression	15
		+936C>T	T allele, lower expression	15
HGF	Role in angiogenesis, tumorigenesis, tissue regeneration	rs5745652, C/T	CC genotype is associated with lower serum HGF levels	16
		rs2074725, C/A	CA and AA genotypes are associated with lower serum HGF levels	16
CYP26B1	Involved in the metabolism of retinoic acid	rs2241057, A/G	G allele associated with higher levels of retinoic acid catabolism and reduced retinoid availability	17
PROX1	Critical role in neurogenesis, development of the heart, eye lens, liver, pancreas and lymphatic system	rs340874, T/C	CC genotype is associated with higher nonesterified fatty acids levels, lower glucose oxidation, higher accumulation of visceral fat	18
RORC	Essential for lymphoid organogenesis	rs11801866, A/T	T allele, higher risk of secondary lymphedema, might affect transcription factor binding sites	19
		rs12128071, G/A	It might affect transcription factor binding sites	19
		rs12045886, A/G	G allele, secondary lymphedema predisposition after breast cancer surgery	19

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Table 1 *(continued)***.** Polymorphisms that can predispose to secondary lymphedema and/or modulated the clinical course of lymphedema

Gene	Gene function (GeneCards)	rs ID, alleles	Association	Ref.
LCP2	T-cell antigen receptor mediated signaling	rs572192, C/T	T allele, secondary lymphedema predisposition after breast cancer surgery	20
		rs6866733, C/G,T	T allele, secondary lymphedema predisposition after breast cancer surgery	20
		rs315721, A/G	AG and GG genotype are associated with a 50% decrease in the odds of developing secondary lymphedema	20
NRP2	It binds interacts with vascular endothelial growth factor (VEGF)	rs849530, G/T	TT and TG genotype are associated with 62% decrease in the odds of developing secondary lymphedema	20
		rs849563, T/A,G	G allele, secondary lymphedema predisposition after breast cancer surgery	20
		rs16837641, G/A,C,T	A allele, secondary lymphedema predisposition after breast cancer surgery	20
SYK	Regulation of innate and adaptive immunity, vascular development. Plays a crucial role in the innate immune response to fungal, bacterial and viral pathogens. Activates the inflammasome and NF-kappa-B-mediated transcription of chemokines and cytokines in presence of pathogens. It is involved in vascular development where it may regulate blood and lymphatic vascular separation	rs158689, T/A	AA and AT genotypes are associated with 3.43-fold increase in the odds of developing secondary lymphedema	20
VCAM1	Pathophysiologic role in immune responses and leukocyte emigration to sites of inflammation	rs3176861, C/T	CT and TT genotypes are associated with a 45.0% decrease in the odds of developing secondary lymphedema	20
miR499	miR-499 gene targets are involved in remodeling and inflammation-related signaling pathways; including fibrogenic and immune-modulator pathways	rs3746444, A/C,G	Associated with inflammatory arthritis susceptibility. The A allele creates an altered target gene set. Disruption of 667 genes of the miR-499a targets and creation of new 763 genes	21
CDKN2B-AS1	Interacts with polycomb repressive complex-1 and -2, leading to epigenetic silencing	rs1333048, A/C,G	AA genotype is associated with elevated C-reactive protein plasma levels	22
CALCRL	Receptor for calcitonin-gene-related peptide together with RAMP1 and receptor for adrenomedullin together with RAMP3 and RAMP2	rs185008808, C/T	Common colds susceptibility	23
		rs61739909, A/G	Waist-hip ratio	23
		rs10177093, G/C,T	Waist-hip ratio	23
VEGFC	Growth factor active in angiogenesis of veins and lymphatics, endothelial cell growth, stimulating their proliferation, migration, permeability of blood vessels	rs2333496, C/T	T allele, waist-hip ratio increase	24
		rs7664413, C/T	T allele, secondary lymphedema predisposition after breast cancer surgery	20

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Table 1 *(continued)***.** Polymorphisms that can predispose to secondary lymphedema and/or modulated the clinical course of lymphedema

Gene	Gene function (GeneCards)	rs ID, alleles	Association	Ref.
EPHB4	Regulation of cell adhesion and migration, angiogenesis, blood vessel remodeling, permeability	rs314313, T/A,C,G	G allele, Crohn's disease/ulcerative colitis/inflammatory bowel disease susceptibility	25
		rs314311, T/G	T allele, low density lipoprotein cholesterol levels decrease	
PLA2G4A	Hydrolyzes arachidonyl phospholipids for releasing arachidonic acid. Implicated in the initiation of the inflammatory response.	rs10798069, G/T	G allele, Crohn's disease/ Inflammatory bowel disease	26
IL1R1	Mediator involved in cytokine-induced immune and inflammatory responses.	rs949963, C/T	A allele, secondary lymphedema predisposition after breast cancer surgery	27
IL4	B-cell activation, DNA synthesis stimulation, expression induction of MHC-II on resting B-cells, secretion enhancement and cell surface expression of IgE, IgG, expression regulation CD23 IgE receptor on lymphocytes and monocytes, expression induction of IL31RA in macrophages, autophagy stimulation in dendritic cells	rs2227284, T/C,G	A allele, secondary lymphedema predisposition after breast cancer surgery	27
IL6	Inducer of the acute phase response, final differentiation of B cells into Ig-secreting cells, lymphocyte and monocyte differentiation, generation of Th17 cells, myokine, increase the breakdown of fats, improve insulin resistance	rs2066992, G/A,C,T	T allele, secondary lymphedema predisposition after breast cancer surgery	27
IL10	Cytokine produced by monocytes, lymphocytes, pleiotropic effects in immunoregulation, inflammation, down-regulation of Th1 cytokines expression, MHC-II, stimulator of macrophages, B cell survival enhancement, proliferation, antibody production	rs1518111, T/C	T allele, secondary lymphedema predisposition after breast cancer surgery	27
		rs1518110, A/C,G,T	A allele, secondary lymphedema predisposition after breast cancer surgery	
NFKB2	Pleiotropic transcription factor ubiquitously expressed involved in inflammation, immunity, differentiation, cell growth, tumorigenesis, apoptosis	rs1056890, G/A,C	A allele, secondary lymphedema predisposition after breast cancer surgery	27
ANGPT2	Endothelial cell migration and proliferation	rs6990020, C/A,T	C allele, secondary lymphedema predisposition after breast cancer surgery	20
SOX17	Embryonic vascular development, postnatal angiogenesis	rs12541742, C/G,T	T allele, secondary lymphedema predisposition after breast cancer surgery	20

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Table 1 *(continued)***.** Polymorphisms that can predispose to secondary lymphedema and/or modulated the clinical course of lymphedema

Gene	Gene function (GeneCards)	rs ID, alleles	Association	Ref.
FLT4	Lymphangiogenesis and maintenance of the lymphatic endothelium	rs75614493, C/T	C, higher risk of secondary lymphedema	11
		rs10464063, A/G	G allele, secondary lymphedema predisposition after breast cancer surgery	19
		rs307814, G/A	A allele, secondary lymphedema predisposition after breast cancer surgery	
		rs307811, C/T	T allele, secondary lymphedema predisposition after breast cancer surgery	
		rs11960332, C/T	T allele, secondary lymphedema predisposition after breast cancer surgery	
		rs11739214, G/C	C allele, secondary lymphedema predisposition after breast cancer surgery	
KDR	Endothelial proliferation, survival, migration, tubular morphogenesis, sprouting.	rs2239702, G/A	A allele, secondary lymphedema predisposition after breast cancer surgery	19
		rs4576072, A/G	G allele, secondary lymphedema predisposition after breast cancer surgery	
		rs10020464, C/A,T	T allele, secondary lymphedema predisposition after breast cancer surgery	20
		rs11133360, C/T	C allele, secondary lymphedema predisposition after breast cancer surgery	
CYP2A6	High coumarin 7-hydroxylase activity	rs1801272, T/A	A allele, significant reduction of coumarin metabolism	28

Inflammation in lymphedema

The fluid accumulation typical of lymphedema stimulates the activation of the inflammatory response. This inflammation modify the extracellular matrix that further decreases lymphatic function (29). Patients with lymphedema are characterized by the upregulation of pro-inflammatory genes (e.g. TNF and IL1). In response to these factors, the dendritic cells synthesize digestive enzymes that allow the passage of dendritic cells through the extracellular matrix into the lymphatic vessels (30). However, in presence of lymphatic injury, dendritic cells concentrate in the site where lymph accumulates. Therefore, they produce additional pro-inflammatory factors that make the inflammation chronic (31). Another typical characteristic of lymphedema is fibrosis. This fibrotic evolution is determined by the synthesis of pro-fibrotic cytokines by Th2 cells, such as IL-4, IL-13 and TGF-β1. These cytokines affect the survival, proliferation and migration of lymphatic endothelial cells (32).

Leukotriene B4 synthesis, function, and its inhibition by hydroxytyrosol

Leukotrienes are derived from the oxidation of arachidonic acid catalyzed by an enzyme called 5-li-

poxygenase (5-LO). This step leads to the formation of the conjugated triene epoxide LTA4. LTA4 is then released by 5-LO and is converted into leukotriene B4 (LTB4) by the enzyme LTA4 hydrolase (LTA4H) (33). LTB4 exerts its biological activity after binding G-protein coupled receptors designated LTB4R and LTB4R2 (34). LTB4 is produced by activated neutrophils and macrophages and has the ability to recruit and activate immune cells. LTB4 at lower concentrations stimulates neutrophil chemotaxis, adherence and migration to venule walls, whereas at higher concentrations stimulates neutrophil lysosomal enzyme release, generation of superoxide radicals, and production of IL-8 and LTB4 (35). Elevated concentrations of LTB4 have been found in secretions in a wide variety of inflammatory conditions including cystic fibrosis, asthma, respiratory distress syndrome, rheumatoid arthritis, inflammatory bowel disease and lymphedema. Excessive neutrophil recruitment and activation by LTB4 may cause tissue damage thereby contributing to the pathological features and progression of lymphedema (36**)**. Interestingly, it was previously shown that the antagonism of leukotriene B4 synthesis or binding to its receptors is able to improve lymphedema *in vitro* in human lymphatic endothelial cells and *in vivo* in mouse model (4).

In humans, HT is able to inhibit the 5-lipoxygenase enzyme activity, thereby blocking leukotriene B4 generation (37). Furthermore, 5-LO is a non-heme iron dioxygenase and HT is able to bind the 5-LO iron ions reducing them to a catalytically inactive ferrous form (38).

Conclusions

Lymphedema is a common disorder with a multifactorial origin. In the recent years, it is becoming more and more clear that genetics play an important role in the pathogenesis and progression of this disorder. Therefore, we think that analyzing polymorphisms that predispose to onset of lymphedema or that could modulate the progression of the disease would be of extreme importance to gain insights into the individual genetic background. This could also be exploited to plan a personalized treatment and management of lymphedema. Additionally, the use of food supplement based on the natural phenol, HT, may help in the treatment of the negative effects of lymph accumulation as we previously reviewed (5).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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